REMARKS

Claim Amendments

Applicants appreciate the Examiner's reconsideration and rejoinder of claims 26-28 with Group I.

Claims 2-3, 8-9, 22-25 and 27-28 have been amended to change "A" to "The" in respect of proper antecedent basis.

Claim 26 has been amended to recite "wherein said third domain does not substantially bind to neuronal cells". Basis for this amendment exists at lines 29-30 on page 15 of the specification.

Claim 27 has been amended to depend from Claim 26.

Claim 38 has been amended to delete the phrase "or a nucleic acid encoding said polypeptide, to a patient".

New Claims 40-44 have been added.

Claim 40 is similar to Claim 1, but recites the additional feature of a third domain, wherein "the third domain targets the agent to a non-neuronal inflammatory cell". Basis for this claim is in previous Claims 1 and 3, and in the specification, page 15, lines 29-30.

Claim 41 is also similar to Claim 1, but recites that the first domain "comprises a light chain of a clostridial neurotoxin, or a fragment thereof, said light chain or fragment having protease activity that cleaves one or more proteins essential to exocytosis". Basis for this claim is in Claims 1 and 23.

Claim 42 is similar to Claim 41, but also recites that the second domain "comprises a H_N domain of a clostridial polypeptide, or a fragment thereof that translocates the first domain into the inflammatory cell". Basis for this claim is in Claims 1 and 23-24.

Claim 43 recites that the first domain "comprises a light chain of a clostridial neurotoxin, or a fragment thereof, said light chain or fragment having protease activity that cleaves one or more proteins essential to exocytosis". Basis for this claim is in Claims 26 and 23.

Claim 44 is similar to Claim 42, but also recites that the second domain "comprises a H_N domain of a clostridial polypeptide, or a fragment thereof that translocates the first domain into the inflammatory cell". Basis for this claim is in Claims 26 and 23-24.

Claim 45 is presented for removal of the parenthesis from claim 9.

Each of the rejections is specifically addressed below.

Objection to claims 2-3, 8-9, 22-25 and 27-28 because "A" should have been "The" for said dependent claims

The Examiner is respectfully requested to remove this objection based upon the amendments to these claims presented above.

Objection to claim 27 as dependent on a canceled claim

The Examiner is also respectfully requested to remove this objection based upon the amendment to this claim presented above.

Objection to claims 8-9 and 38 as encompassing non-elected embodiments

With regard to claim 8, Applicants respectfully point out that in the Response to Restriction Requirement mailed December 30, 2004, Applicants elected the species wherein the third domain is IL-8. However, and as set forth more fully below, all the species of claim 8 are fully embraced by an allowable generic claim. Therefore, Applicants respectfully request the Examiner to withdraw the stated objection.

With regard to Claim 9, Applicants presume the Examiner's rejection is based upon the assumption that at least some of the recited diseases are not caused, exacerbated or maintained by secretion from non-neuronal inflammatory cells. In response, Applicants refer the Examiner to the enclosed references appended hereto as "Exhibit A" which confirm a direct link between non-neuronal inflammatory cells and the conditions recited in Claim 9. In particular, it is evident from these references that the recited conditions are caused, exacerbated or maintained by secretions from non-neuronal inflammatory cells. For the Examiner's convenience, portions of each reference are highlighted. Applicants respectfully request the Examiner to remove the objection to claim 9 in view of these references.

With regard to the objection to Claim 38, this claim has been amended by deleting reference to nucleic acids. Thus, the Examiner's objection is no longer applicable and Applicants request the Examiner to remove the stated objection.

Rejection of Claims 1-3, 8-9, 22-28 and 38 under 35 U.S.C. 112, first paragraph (alleged lack of enablement)

The Examiner has argued the specification does not enable one skilled in the art to make and use the invention commensurate in scope with these claims, and that an undue amount of experimentation would be required for one skilled in the art to practice the invention as claimed. In explanation of this rejection, the Examiner essentially argues that the specification only discloses a polypeptide (LH_N--WGA) comprising a first domain, a second domain and a third domain, where the first domain is a light chain of botulinum neurotoxin containing endopeptidase activity specific for a protein selected from the group consisting of SNAP-25, synaptobrevin and syntaxin covalently linked to a second domain wherein the second domain is a clostrial toxin heavy chain H_N covalently linked to a third domain wherein the third domain is wheat germ agglutinin. The Examiner also alleges that the specification only discloses a method

of inhibiting secretion of histamine from human umbilical vein endothelial cells in vitro. Based on this, the Examiner asserts that the specification does not teach how to make any or all of the claimed "agent." The Examiner has also argued there is insufficient guidance as to how to use such an agent for treating the diseases recited in claims 9 and 36. For the following reasons, Applicants respectfully disagree.

Prior to the present invention, it would have been routine for a skilled person to generally prepare agents having first, second and third domains. In this regard, conventional techniques suitable for preparation of the presently claimed agents are described on pages 18-23 of the present description. These techniques include direct linkage, linkage via a suitable spacer/ linker molecule (see lines 3-6, page 18 of the specification), or recombinant preparation (see pages 21-22). Basic conjugation and coupling techniques are also exemplified in Examples 1 and 5 of the present specification, although any conventional conjugation or coupling chemistry may be employed to prepare an agent according to the present claims.

By way of example, as described on page 18, line 21 to page 19, line 2, a complex may be formed of first and second (E and T) domains, which is then mixed with a reduced third domain (B) to form a disulphide-linked agent having all three components. Alternatively, as described in lines 4-29 on page 19, a derivatized third domain (B) may be mixed with a first-second (E-T) domain complex, to form a covalently-coupled agent having all three components. It would also have been routine for a skilled person to employ a spacer. The present application discloses examples of suitable spacers for use with the invention, such as those provided on page 20, line 5 - page 21, line 2, to link a B domain to an E-T complex (see lines 5-11 on page 20); or to link each domain together (see page 21, lines 4-23).

The above-mentioned general methodologies are further exemplified in specific embodiments of the present application. By way of example, in one embodiment, the E-T complex may be an LH_N component of a clostridial neurotoxin, which may then be linked to any suitable targeting domain (TM) (see page 16, lines 3-9, of the specification). Alternatively, the L chain of one clostridial neurotoxin may be complexed with the H_N domain of a different clostridial neurotoxin, prior to complexing with a suitable TM (see lines 26-29 on page 16). Furthermore, prior to the present invention, all the components of the presently claimed agents were commercially available or could otherwise routinely be isolated, and their sequence information could be obtained from known publications (see, for example, pages 22-24 of the specification.)

Additional objective evidence that the presently claimed agents can be made and have the desired activity is provided by the enclosed Rule 1.132 Declarations (originally prepared in connection with another Health Protection Agency application, U.S. Serial No. 09/529,130; Declarations attached as "Exhibit B") by Dr. John Chaddock and Dr. Keith Foster. Specifically, the Declarations describe a range of different chemical coupling agents that may be used to link one or more domains of an agent according to the present invention including, but not limited to, SPDP, PDPH/EDAC, Traut's Reagent, SMPB and SMCC. The Declarations also describe a number of routine tests that a skilled person would employ to confirm that an agent of the present invention has the requisite biological activity.

The Examiner has also alleged that a skilled person reading the present specification would not be able to use the recited agents to treat the specific diseases recited in Claim 9 (and embraced by Claim 38), i.e. diseases caused, exacerbated, or maintained by secretion from a non-neuronal inflammatory cell and treatable using a polypeptide that cleaves one or more proteins essential to exocytosis. However, Applicants respectfully disagree, since it would be apparent to a skilled person that the presently claimed agents would be suitable for treating the recited conditions. Applicants have provided herewith three references (Pinxteren *et al.*, 2000; Specht *et al.*; and Guo *et al.*; copies attached as "Exhibit C") emphasizing this point as explained more fully below.

Efficacy of the claimed agents stems from delivery of the first "endopeptidase" domain into a desired target cell. By way of example, Pinxteren *et al.*, 2000 (cited in the present specification, page 14), describes inhibition of secretion from permeabilized eosinophils in the presence of botulinum C1 holotoxin (see Figure 4, page 390). In this regard, the first "endopeptidase" domain (having exocytosis-inhibitory activity) is inherent to holotoxin. Thus, Pinxteren *et al.*, 2000 confirms that a clostridial neurotoxin L-chain, once delivered to a target cell (e.g. an eosinophil), has efficacy for treating a condition associated with excessive secretion.

Targeting of an agent to a desired cell-type is routinely achieved by linkage to a TM that binds to that specific cell-type. In this regard, the enclosed Specht *et al.* abstract (abstract No. 138 on page R43) describes the use of IgE as a TM for targeting to mast cells. Thus, Specht *et al.* confirms that an agent of the present invention, comprising a clostridial neurotoxin light chain as a first domain and a clostridial neurotoxin H_N component as a second domain, can be targeted to a desired inflammatory cell-type, such as mast cells.

Following delivery to a desired target non-neuronal inflammatory cell by an appropriate TM, the agents of the present invention exert their effect by cleaving one or more exocytic proteins located in that cell. In this regard, Guo *et al.*, 1998, confirms that the exocytic protein substrates on which the presently claimed agents act, such as SNARE proteins including SNAP-25, synaptobrevin/ VAMP and syntaxin, are found in inflammatory cells such as mast cells.

Thus, these publications confirm that the presently claimed agents are suitable for treating the recited conditions because they can be targeted to non-neuronal inflammatory cells (e.g. mast cells), and once targeted, will cleave exocytosis-associated molecules found in these cells, thus resulting in inhibition of secretion from those cells. Furthermore, a skilled person would understand that, by inhibiting secretion from non-neuronal inflammatory cells, the present agents can be used to treat diseases caused, exacerbated, or maintained by secretion from a non-neuronal inflammatory cell.

Therefore, regarding the Examiner's rejection of Claims 1-3, 8-9, 22-28 and 38 under 35 U.S.C. 112, first paragraph, Applicants submit that the present specification clearly teaches the skilled person that the presently claimed agents can be used in the presently claimed methods. The present specification also clearly demonstrates that the claimed agents can routinely be made in view of the teachings therein. Moreover, the applicants have provided additional objective evidence that such conjugates can routinely be made. Thus, Applicants submit the present specification fully enables the pending claims and respectfully request the Examiner to withdraw

the stated rejections.

Rejection of Claims 1-3, 8-9, 22-28 and 38 under 35 U.S.C. 112, first paragraph (alleging lack of written description)

The Examiner has raised a written description objection, alleging that the disclosure is not representative of all possible agents that fall within the scope of the pending claims. Applicants respectfully disagree. The agents of the present invention may comprise any active component that cleaves one or more proteins essential to exocytosis (ie. vesicle/ plasma-membrane associated proteins), any "translocation" domain that translocates the active component into a target cell, and any targeting moiety (TM) that binds to the surface of a non-neuronal inflammatory cell. Further discussion of each domain follows below.

Regarding the First domain

As described on page 9, lines 1-30, of the present specification, the first domain comprises any polypeptide that cleaves one or more proteins essential to exocytosis (see lines 7-12 on page 8 of the specification). In more detail, the first domain is an endopeptidase polypeptide that cleaves an exocytosis protein selected from SNAP-25, synaptobrevin/ VAMP and syntaxin (see lines 25-27 on page 9 of the specification). By way of example, said first domain may be the light chain of a clostridial neurotoxin, e.g. the L-chain of botulinum or tetanus toxin (lines 2-3, page 2). The agents of the present invention may, however, include other endopeptidases that also cleave SNAP-25, synaptobrevin/ VAMP or syntaxin. By way of example, we refer to the non-cytotoxic toxin I_gA protease of Neisseria (in particular, from the species N. gonorrhoeae), which exerts its effect by cleaving exocytosis-related proteins such as SNAP-25, synaptobrevin/ VAMP or syntaxin (see the enclosed publication CA2331274 included in Exhibit C).

Thus, it would be apparent to a skilled person reading the present specification that any molecule having the defined endopeptidase activity would be suitable for use as a first domain in the presently claimed agents. Furthermore, a skilled person would conclude that the disclosure of the specification provides a written description of a representative number of species of first domains to describe the claimed genus.

Furthermore, it would be routine for a skilled person to confirm that a first domain of an agent of the present invention has the desired biological activity, by carrying out a simple test for endopeptidase activity, such as one of the conventional tests described in the enclosed Declarations of Drs. Foster and Chaddock.

Regarding the Second domain

As described in lines 8-10 on page 8 of the, specification, the second domain comprises a polypeptide that translocates the agent into the target cell (or at least that part of the agent responsible for inhibiting exocytosis). One example of a second domain (ie. translocation domain) comprises a clostridial toxin heavy chain H_N portion, or fragment/ variant thereof (see

the passage spanning pages 9-10 of the specification). In this regard, the second domain may comprise a heavy chain H_N portion from a botulinum neurotoxin (serotypes A, B, C, D, E, F or G) or tetanus neurotoxin; or may be derived from C. butyricum or C. argentinense toxins (see lines 11-24 on page 17 of the specification). Furthermore, the present specification also provides examples of a range of non- clostridial sources of translocation domains, including diphtheria toxin, pseudomonas exotoxin A, influenza virus haemagglutinin fusogenic peptides, and amphiphilic peptides (see paragraph spanning pages 17-18 of the specification).

Hence, it would be apparent to a skilled person reading the present specification that any molecule having the desired translocation activity would be suitable for use as a second domain in an agent of the present invention. Furthermore, a skilled person would conclude that the disclosure of the specification provides a written description of a representative number of species of second domains to describe the claimed genus. Additionally, it would be routine for a skilled person to confirm that a second domain of an agent of the present invention has the desired biological activity by carrying out a simple test for translocation activity, such as one of the conventional tests described in the enclosed declarations of Drs. Foster and Chaddock.

Regarding the Third domain

The third domain (ie., the TM) is responsible for targeting the agent to the correct cell type. Suitable TMs for use in an agent of the present invention include the numerous different polypeptides recited on page 12, lines 11-24, of the present specification (see also present Claim 8), which all bind to a binding site on a non-neuronal inflammatory cell. As discussed on page 15, lines 14-20, of the present specification, the TM component of the agent may comprise one of many different cell-binding moieties, including antibodies and fragments thereof, lectins, hormones, cytokines, growth factors, peptides, carbohydrates, lipids, glycons, nucleic acids or complement components. Thus, a skilled person reading the present specification would understand that any molecule capable of binding to a non-neuronal inflammatory cell and which does not substantially bind to neuronal cells is suitable for use as a TM in an agent of the present invention. Furthermore, a skilled person would conclude that the disclosure of the specification provides a written description of a representative number of species of third domains to describe the claimed genus. In addition, it would be routine for a skilled person to confirm that a third domain of an agent of the present invention has the desired biological activity, by carrying out a simple test for targeting activity, such as one of the conventional tests described in the enclosed declarations of Foster and Chaddock, and therefore no undue experimentation is required.

Regarding the Production of agents

A range of techniques suitable for preparing agents of the present invention, having first, second and optional third domains, are described on pages 18-23 of the present description. In particular, the polypeptide domains may be joined together by direct linkage or via a suitable spacer/ linker molecule (see lines 3-6 on page 18 of the specification); or the agents may be prepared recombinantly (see passage spanning pages 21-22 of the specification).

By way of example, as described on page 18, line 21, and page 19, line 2, a complex may

be formed of first and second domains, which is then mixed with a reduced third domain to form a disulphide-linked agent having all three components. Alternatively, as described in lines 4-29 on page 19, a derivatized third domain may be mixed with a first-second domain complex, to form a covalently-coupled agent having all three components.

A range of suitable spacer molecules are provided on page 20, line 5 and page 21, line 2. Spacers may be used between the third domain and a first-second domain complex (see lines 5-11 on page 20); or may be used between each domain (see page 21, lines 4-23).

The above-mentioned general methodologies are further exemplified in specific embodiments of the present application. By way of example, in one specific embodiment, the first-second domain complex may be formed by removing the H_c domain from a clostridial neurotoxin, to leave the disulphide-linked LH_N domain, which may then be linked to any suitable TM (see page 16, lines 3-9, of the specification). Alternatively, the binding activity of the H_c domain may be inactivated by mutation. Furthermore, the L chain of a clostridial neurotoxin may be complexed with the H_N domain of a different clostridial neurotoxin, e.g. from a different serotype or different species, prior to complexing with a suitable TM (see lines 26-29 on page 16). If the selected TM comprises translocation activity, a second domain may not be required, in which case, in a specific embodiment, the L chain of a clostridial neurotoxin may be linked to a TM in the absence of a H_N domain (see passage spanning pages 16-17 of the specification).

The use of these techniques (together with tests for identifying the presence of suitable first, second and third domains) is confirmed by the enclosed Declarations as discussed in more detail above with regard to enablement.

Therefore, regarding the Examiner's rejection of claims 1-3, 8-9, 22-28 and 38 under 35 U.S.C. 112, first paragraph, Applicants respectfully submit that it would be apparent to a skilled person that the disclosure of the present specification provides a written description of a broad range of species of agents falling within the scope of the genus recited in the pending claims. The specific conjugate the Examiner has indicated as disclosed by the present specification (LH_N— WGA) is but one species of the presently claimed genus. Therefore, in contrast to the Examiner's assertion, the Applicants were in possession of the invention across the entire scope of the pending claims and respectfully request that the Examiner's rejection of the claims be withdrawn.

Rejection of claims 1-3, 9, 22-24 and 26-28 under 35 USC 102(b)

The Examiner has acknowledged novelty of Claims 8, 25 and 38, but has alleged that the remaining claims lack novelty in view of cited publication WO 98/07864, which allegedly describes an agent that is capable of binding both to neuronal cells and to non-neuronal cells. However, as amended herewith, Claim 26 recites that the third domain (and hence, the "agent") does not substantially bind to neuronal cells. Thus, Claims 26-28 and 43-44 are novel over WO 98/07864. Method Claims 1-3, 8-9, 22-24 and 40-42 are also novel over WO 98/07864, which fails to disclose any method of inhibiting secretion from a non-neuronal inflammatory cell. Applicants therefore request the Examiner to withdraw the stated rejections.

Rejection of claims 1-3 and 8 under 35 USC 103(a)

The Examiner has argued that Claim 8 is obvious to the extent to which the third domain is IL-8. The Examiner's allegation is made in view of WO 98/07864 (which allegedly discloses an agent wherein the third domain is IGF-1) in combination with WO 96/33273 (which allegedly discloses an agent wherein the third domain is IL-8) and Van Damme *et al.* (which allegedly discloses a link between IL-S secretion and inflammation).

However, Applicants respectfully submit that a skilled person seeking to modify the agents of WO 98/07864 in order to create an agent that inhibits secretion from a non-neuronal cell would not turn to WO 96/33273, which relates solely to targeting neuronal cells. As herein amended, the claims recite an agent having a third domain that does not substantially bind to neuronal cells. Such agents would not be obvious from WO 98/07864 or WO 96/33273, and hence the present claims are not obvious in view of these citations.

Applicants further point out that a skilled person would not combine WO 96/33273 with Van Damme *et al.*. Specifically, WO 96/33273 relates solely to targeting BoNT activity to neuronal cells, whereas Van Damme *et al.* is directed towards neutrophils, which are non-neuronal cells. WO 96/33273 provides no suggestion to a skilled person that BoNT activity should, or even could, be re-targeted to non-neuronal cells such as neutrophils. In particular, there is no suggestion in WO 96/33273 that BoNT would have any activity in non-neuronal cells. Thus, the present invention is not obvious in view of a combination of WO 96/33273 with Van Damme *et al.*

The present invention is also not obvious over a combination of WO 98/07864 (a prior patent application in the name of the present inventors) with Van Damme *et al.* In particular, the WO 98/07864 agents all have third domains that bind to neuronal cells (e.g. IGF-1), and hence WO 98/07864 describes agents that target neuronal cells. This is at odds with the disclosure of Van Damme *et al.*, which relates to non-neuronal cells (neutrophils), and hence a skilled person would not combine the teaching of these two documents. Furthermore, the problem addressed by WO 98/07864 (reduction of agent toxicity) is entirely distinct from that addressed by Van Damme *et al.* (further characterizing the role of IL-8 in neutrophils). Further, both of these problems are remote from the problem addressed by the present invention, namely, the provision of an agent that inhibits secretion from a non-neuronal inflammatory cell.

Furthermore, even if WO 98/07864 and/or WO 96/33273 were combined with Van Damme *et al.*, this combination would fail to suggest the presently claimed invention. Indeed, Van Damme *et al.* teaches away from using IL-8 as a third domain in an agent according to the present invention. Specifically, Van Damme *et al.* describes the pro-inflammatory effects of IL-8 (see the 2nd sentence of the Introduction, and first sentence of the Discussion). In this regard, IL-8 is a potent pro-inflammatory cytokine having chemoattractant properties for granulocytes. In other words, the binding of IL-8 to inflammatory cells induces an inflammatory response. IL-8 was initially identified due to its neutrophil activating and chemoattractant properties, and increased IL-8 expression is seen in allergic inflammation. Thus, a skilled person reading Van

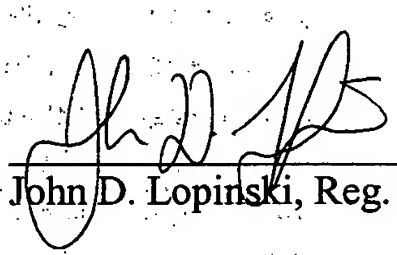
Damme *et al.* would seek to inhibit IL-8 binding to target cells, and would not consider IL-8 to be a suitable targeting moiety for an anti-inflammatory agent of the present invention. Further evidence of the pro-inflammatory activity of IL-8 is provided by the Abstracts in Exhibit D appended hereto. Thus, the agents of Claim 8, in which the third domain is IL-8, are not obvious over WO 98/07864, WO 96/33273 and Van Damme *et al.*, and Applicants respectfully request that these rejections be withdrawn.

CONCLUSION

Applicants believe that based on the amendments presented and arguments presented herein, all the pending claims are now in a condition for allowance and respectfully request the Examiner to allow these claims.

Applicants herewith file a request for a one-month extension to reply and the required fee of $120. If any additional fee is due, the Examiner is authorized to charge it to Deposit Account No. 08-2442.

Respectfully submitted,



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